IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	**10/621,760**
Applicants	:	**David L. Lewis et al.**
Filed	:	**07/17/2003**
Art Unit	:	**1633**
Examiner	:	**Popa, Ileana**
Docket No.	:	**Mirus.030.09.2**

For: **Compositions and Processes Using siRNA, Amphipathic Compounds and Polycations**

Commissioner of Patents
PO Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. ' 1.132

Dear Commissioner

I, James E. Hagstrom, hereby declare as follows:

1. I am an inventor of the above captioned application.
2. I have submitted, with the amendment filed 12-5-2007, a Declaration containing experimental material illustrating: delivery of siRNA to mammalian cells using a complex consisting of an amphipathic compound, DNA and either histone or ethoxylated polyethyleneimine.
3. The complexes were prepared as taught in Wolff et al. (U.S. Patent 5,744,335).
4. The amphipathic compound of the Declaration experiments was identical to that used by Wolff et al. (U.S. Patent 5,744,335).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

12/5/07

Dr. James E. Hagstrom Date

Cos7 cells, 293 cells, or 3T3 cells were initially transfected with a plasmid DNAs encoding the Firefly luciferase gene and the Renilla luciferase genes, resulting in cells that expressed these two luciferase proteins. These cells were then transfected with 5 nM Firefly luciferase siRNA using either ethoxylate polyethyleneimine (ePEI) + amphipathic compound or histone + amphipathic compound.

Successful delivery of the Firefly luciferase siRNA to cells expressing the Firefly luciferase gene results in knockdown (inhibition) of Firefly luciferase gene expression. Thus, delivery of Firefly luciferase siRNA directly correlates with knockdown of Firefly luciferase gene expression. Higher knockdown means more efficient delivery, while lower knockdown means less efficient or no delivery. As shown in the table below, siRNA was efficiently delivered with ePEI + amphipathic compound: 71% to 90% knockdown. In contrast, histone + amphipathic compound was not an effective siRNA delivery reagent: 0% knockdown.

The histone + amphipathic compound was the same composition as that used in U.S. Patent 5,744,335. The ePEI + amphipathic compound was the same as that described in Application 10/621,760. The amphipathic compound used with histone was the same as that used with ePEI.

transfection reagent	Luciferase expression	% knockdown
none	1.0000	0.0000
ePEI + amphipathic compound	0.0960	0.9040
histone + amphipathic compound (ratio 1)	*1.0760*	*−0.0760*
histone + amphipathic compound (ratio 2)	*1.1820*	*−0.1820*
histone + amphipathic compound (ratio 3)	*1.1980*	*−0.1980*
ePEI + amphipathic compound	0.2830	0.7170
histone + amphipathic compound	*1.0398*	*−0.0398*
ePEI + amphipathic compound	0.2222	0.7778
histone + amphipathic compound	*1.0344*	*−0.0344*